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14. ABSTRACT: The disproportionate incidence and mortality of prostate cancer (CaP) among African Americans (AA) in comparison to Caucasian American (CA) are not well understood. It is believed that high circulating androgens reported in AA men may account for such racial disparities. It has been shown that metastatic tumors maintain functional androgen receptor signaling, suggesting that local (intracrine) androgens may contribute to the outgrowth of 'castration-adapted' tumors under androgen deprivation therapy (ADT). Evidence exists for direct correlation between increased obesity and body-mass-index (BMI), which is significantly higher in AA-men, and the risk for aggressive CaP. Active steroidogenic pathways are active in adipocytes and adipose-derived mesenchymal stem cells (ASCs) are often recruited to tumor-stroma. Our goal will be to exploit the tumor-tropism of normal ASCs to deliver androgen inactivating genes to tumor microenvironments and enable an effective treatment strategy against CRPC. This will be achieved by: (a) investigate if "intracrine" production of testosterone by osteotropic ASCAA modulates growth and metastatic potential of CaP cells under ADT in vitro and in vivo; (b) determine if α-HSD-expressing osteotropic ASCCont will nullify the ADMSCAA-mediated CaP cell growth and metastasis in vitro; and (c) examine the efficacy of therapeutically engineered ASCCont to target and inhibit CaP tumor growth under CRPC in vivo. The proposed work will be innovative, because it capitalizes on an adjuvant approach for ADT by tumor-site specific inactivation of androgens. Considering the aggressive nature CaP, the outcome of our study is expected to have a positive impact on establishing preventive and/or therapeutic intervention strategies to reduce or circumvent PC, especially among AA-men.

15. SUBJECT TERMS
Prostate cancer, health disparity, stem cells, hormone inactivating enzymes, CRPC, steroidogenic enzymes, African Americans.

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Introduction:

Race, a Risk Factor for Prostate Cancer (CaP). In the US, African Americans have the highest annual incidence of CaP, at 272 new cases per 100,000 men [1]. In 2009, 230,000 men were diagnosed with CaP, and more than 38,000 afflicted men are expected to die [2]. Although the etiology remains largely unknown, racial make-up has been identified as one of several risk factors for CaP. African-American (AA) men bear a disproportionately heavy burden from this disease with incidence and mortality rates over 50% higher than Caucasian American (CA) men [3, 4]. Furthermore, AA men are more likely to develop CaP at an earlier age, have higher rate of Gleason-7, aggressive tumors, and metastasis, and exhibit a poorer survival rate than CA males [3-7]. Socioeconomic and environmental factors, such as diet, access to care, and screening, have been cited as factors contributing to the more clinically aggressive CaP in AA patients [8, 9]. Family history accounts for 5-10% of total CaP cases [8, 9], and it does not differ among AA, Asian Americans, and CA men [10, 11]. A more biologically aggressive CaP has been proposed as one possible explanation for the younger age at presentation and disease progression in AA men compared with CA men [12, 13].

Obesity and BMI as Risk Factors for CaP progression in AA men. Prostate cancer incidence and mortality rates correlate well with the average intake of fats, including polyunsaturated fats [14]. *In vivo* and *in vitro* models have demonstrated a decreased rate of proliferation of prostate tumors with reduced fat intake [15-17]. One meta-analysis showed a 5% excess risk of developing prostate cancer for each 5 kg/m^2 increment of BMI [18]. When disease stage was considered, the analysis showed a rate ratio for advanced cancer of 1.12 per 5 kg/m^2 increment. An analysis from the CaP Prevention Trial noted that compared with men with a BMI below 25 kg/m^2, those with a BMI above 30 kg/m^2 had an 18% decrease in the risk of low-grade cancer, but a 29% increase in the risk of high-grade cancer [19]. In addition, obesity has been linked to aggressive CaP [20] and increased BMI has also shown a positive correlation to Gleason score and positive surgical margins [21]. The latter is critical as it can be an indicator for disease relapse. However, the mechanisms linking obesity to CaP development and progression are not fully understood. Since the prevalence of obesity is significantly high in AA men [22], accounting for 37.3%, unraveling such mechanisms is of paramount significance.

"Intracrine androgens" and CaP progression. Androgen-deprivation therapy (ADT) has been the mainstay treatment for patients with metastatic CaP [23]. Although initially effective, hormonal therapy is marked by progression to castration-resistant prostate cancer (CRPC) over a period of 18–20 months, with median survival of 1–2 years. Importantly, large body of evidence indicate that in the setting of 'castrate' serum testosterone levels, prostatic androgen concentrations remain at approximately 10–25% of the levels found in untreated patients [24-26] well within the range capable of mediating continued androgen-receptor (AR) signaling and gene expression [27]. Moreover, residual intra-prostatic androgens are implicated in nearly every mechanism whereby AR-mediated signaling leads to the development of castration-resistant disease [28]. The increased expression of androgen-metabolizing genes within castration-resistant metastatic tumors [29] strongly suggests that up-regulated activity of endogenous steroidogenic pathways is driving the outgrowth of 'castration-adapted' tumors. The source of residual androgens within the prostate tumors of castrate men has not been fully elucidated, but has been attributed to the uptake and conversion of circulating adrenal androgens [30]. Whether the de novo biosynthesis of androgens from cholesterol or earlier precursors occurs within castration-resistant metastases is not known [28] but has significant implications for treatment strategies targeting sources of androgens exogenous to the prostate versus 'intracrine' sources active within the actual metastatic tumor microenvironments.

Body

Specific Aim-1:- ***Investigate if "intracrine" production of testosterone by enriched ASC^{AA} modulates growth and metastatic potential of CaP cells under ADT in vitro and in vivo*.**

Task-1. Generate a large number of well characterized ADMSC stocks from multiple donors (Months 1-6).

1.1. *Obtain lipoaspirates from normal donors and isolate and culture ASCs by using protocols already established in our laboratories.*
1.2. *Analysis of stem cell characteristics in ASCs by flow cytometry and lineage-specific differentiation, and development of large batches of liquid-N_2 stocks for future experiments.*

Over the past year we have established and stocked ADMSC isolates from African Americans (AA) and Caucasian Americans (CA). All information on research subjects is confidential and the study was conducted in accordance with an approved by an IRB protocol. Upon signing informed consent forms intra-abdominal adipose tissues were procured from the space of Retrius near the dome of the bladder from PC patients undergoing radical prostatectomy at Tulane University Hospital and Clinics, New Orleans, LA. Fat tissue was collected from at least 15 African Americans and Caucasian Americans PC patients with a mean preoperative PSA of 10.5 ng/ml, average age of 59.5 years, average Gleason score of 7.2 and average BMI of 32.4. Normal adipose tissue derived stem cells (nASCs) were generously provided by Dr. Jeffery M. Gimble (Pennington Biomedical Research Center, Baton Rouge, LA).

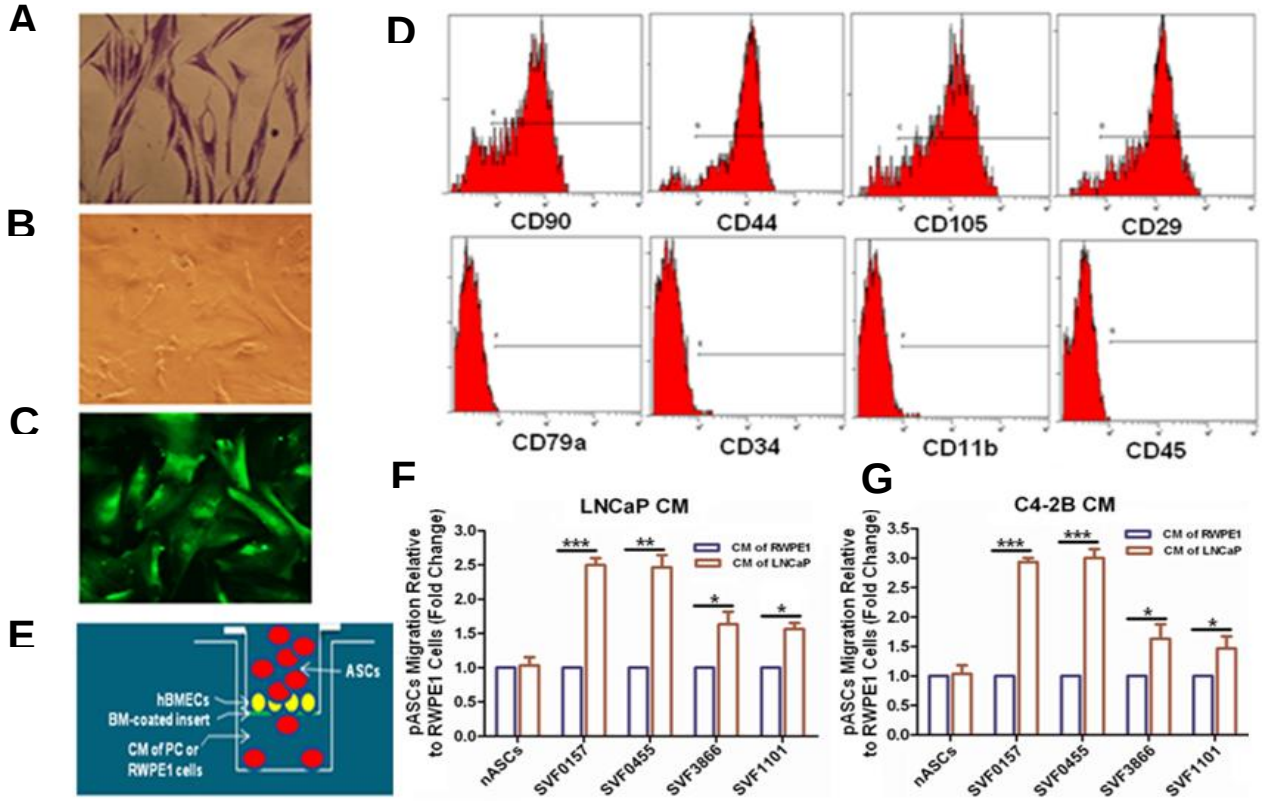

Figure 1. Patient-derived ASCs (pASCs) isolation, characterization and transendothelial migration (TEM) towards PC cell soluble factors *in vitro*. (**A**) Representative photomicrographs (*40 x*) of methylene blue stained pASC isolates with fibroblast-like phenotype. (**B**) pASCs under bright field. (**C**) ASCs stably transduced with pLV-eGFP, a lentivirus construct expressing green fluorescent protein. (**D**) Purity of isolated pASCs was verified by FACS analysis of mesenchymal surface expression markers CD90, CD44, CD105, and CD29 (upper panel) and hematopoietic lineage markers CD79α, CD34, CD11b and CD45 (lower panel). (**E**) Schematic illustration of the TEM system. Human bone marrow endothelial cells (hBMECs) were cultured onto basement membrane (BM)-coated inserts in the upper chamber and CM derived from PC or RWPE1 cells were placed in the lower chamber. (**F**, **G**) TEM of pLV-eGFP-labeled of nASCs or pASCs (1 x 10^5), designated as (SVF), through a confluent layer of hBMECs/BM barrier towards CM of PC or RWPE1 cells in the lower chambers were monitored after 24 hrs by a fluorescence plate reader. Migration in quadruplicates is expressed as a fold change in fluorescence intensity in pASCs towards PC cells after normalization to control ASCs (nACSs) by three independent experiments. * $p<0.05$, ** $p<0.01$ and *** $p<0.00$.

The ASCs were isolated from PC patients (pASCs). Briefly, fresh fat tissue (~ 1 gm) was collected, washed three times in PBS, minced on ice into ~ 1 mm^3 pieces. The minced tissue was suspended in 2 mg/ml of collagenase type-I (GIBCO, Invitrogen, Carlsbad, CA) dissolved in PBS containing 5 mM calcium chloride and subsequently incubated at 37°C in a shaking water bath for 2 hr. To remove tissue debris, the cell suspension was successively filtered through 70 µm and 40 µm cell strainer (BD Biosciences, MD). Mature adipocytes were removed by centrifugation (1,500x*g* for 10 min) followed by washing in PBS. The resulting stromal vascular fraction (SVF) pellet was resuspended and incubated for 2 min in lysis solution (0.15 M ammonium chloride, 10mM potassium bicarbonate and 0.1 mM EDTA) to remove RBCs. Stem cells were washed in 2 ml 1% BSA (Sigma-Aldrich, MO), resuspended in DMEM/F12 medium (1:1; v/v) supplemented with 10% FBS and 1% antibiotics-antimycotic solution (penicillin G, streptomycin and amphotericin B; Mediatech, Herndon, VA) and maintained at 37^oC in an air incubator supplied with 5% CO_2.

The purity of isolated ASCs was verified by FACS analysis as shown in Figure 1 (above). Briefly, cells (2 x10^6) were aliquoted, resuspended in 1 ml of PBS and incubated in the dark for 20 min at room temperature with one of the following antibodies: CD44-APC and CD29-peCy5 (BD Biosciences, MD), CD90-Pe-Cy5, CD105-PE, CD34-PE, CD45-PeCy7, CD79a-PeCy5 and CD11b-peCy5 (Beckman Coulter, CA). One cell aliquot was used as isotype control IgG1/IgG2a and another was unstained. All cells were washed thrice by PBS and resuspended in 0.5 ml PBS, gently vortexed, and then analyzed by Beckman-Coulter Galios 2 Laser, 8 channel flow cytometer running Galios software for acquisition (Center for Stem Cell Research and Regenerative Medicine, Tulane University).

Next, we stably transduced ASCs with pLV-eGFP, a lentivirus construct expressing green fluorescent protein. To isolate tumor-tropic ASCs, we employed a transendothelial migration (TEM) system to enrich for ASCs migration towards PC cell soluble factors *in vitro* (Fig 1). The TEM ability of patient derived ASCs (designated as SVF) towards PC condition media was significantly higher, especially among AA patients' isolates, compared to normal counterparts (Fig. 1).

<u>Task-2</u>. Compare the *in vitro* effects of CaP cells on androgen production by ASCs and determine the effects of ADMSC cocultures on CaP cell growth and metastasis. (Months 2-10)

2.1. *Harvest and freeze aliquots of charcoal-stripped conditioned medium (CM) from different CaP cell lines, e.g. LNCaP, C4-2B, PC-3, MDA-PCa-2a and MDA PCa-2b cell lines.*

We have prepared condition media (CM) from different prostate cancer cell lines. Briefly, cells were cultured in complete media until ~ 70% confluency. After washing (3x in BPS), cells were grown in serum-free media for 24 hrs. After centrifugation (1,000xg, 10 min) and filtration (0.2 µM), the CM were frozen at -20^oC until used.

2.1. *Expose ASC^{Cont}, ASC^{CA} and ASC^{AA} cells to CM from the above CaP cells and measure testosterone (T) and dihydrotestosterone (DHT) levels in culture media by ELISA assays.*

2.2. *Obtain total RNA and proteins from ASCs, control and CaP CM-exposed, and determine the change in expression of different steroidogenic enzymes by qRT-PCR and Western blot.*

Control serum-free medium, CM (1:1; *v*/*v*) or MVs (5-10 µg/mL) derived from RWPE1 or PC cells were added to nASCs or pASCs (70% confluent) pre-cultured in medium supplemented with 10% charcoal-stripped FBS and subsequently harvested at various time frames (up to 96 hr). The expression of AME in ASCs was analyzed by qRT-PCR. The release of androgens in media was measured by an ELISA kit as per manufacturer's recommendations (Cayman Chemicals, Ann Arbor, MI). The sequences of AME amplimer sets used in qRT-PCR analysis are listed in Table 1. The results were normalized to controls and data was expressed as fold change ± SE from three independent experiments.

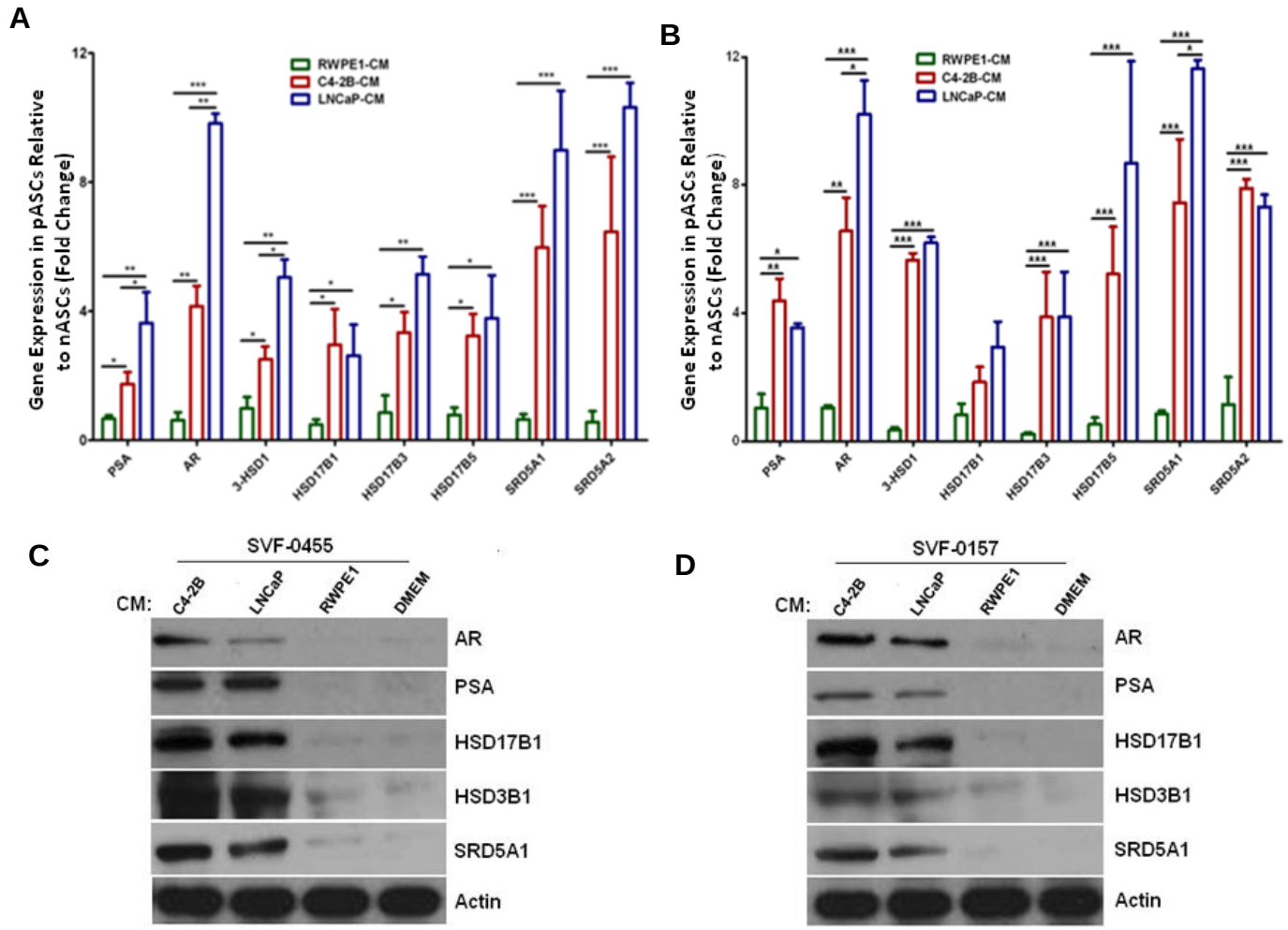

Figure 2. PC cells confer transcriptional upregulation of AR, PSA and steroidogenic gene expression in pASCs *in vitro.* Real-time PCR analysis of gene expression of AR, PSA, AME in pASCs cells, designated SVF0157 **(a)** or SVF0455 **(b),** incubated with CM derived from C4-2B, LNCaP or RWPE1 cells for 24 hrs. Prostate specific markers and AMEs were significantly upregulated in pASCs exposed to CM from C4-2B or LNCaP cells, as opposed CM from RWPE1 cells. Data are expressed as fold change ± s.e.m. after normalization to nASCs (*n*=3). *, ** and *** denotes significance at p<0.5, p<0.01 and p<0.001, respectively. **c, d**, Immunoblot analysis of AR, PSA and AME protein levels in two representative pASCs (SVF0157 and SVF0455) exposed to growth media alone (DMEM) or to CM (50%) from C4-2B, LNCaP or RWPE1 cells for 24 hr.

While morphology remained unchanged, the condition medium (CM) of LNCaP and CB-2B stimulated the growth of enriched pASCs (SVFA122 and VF-0455) in a concentration dependent manner (*data not shown*). The growth induction was associated with transcriptional upregulation of AR (3 to 6-fold) and PSA (2 to 3.5 fold) by PC cell CM in pASCs, derived from AA men, compared to RWPE1 cell CM or control medium (Figure 2). Next, we examined the expression of androgen metabolizing enzymes (AME) involved in *de novo* biosynthesis of testosterone (T). PC cell CM treated pASCs (SVF0157, SVF0455), but not nASCs, induced gene expression of 3-β-hydroxysteroid dehydrogenase (3-β-HSD) and 17-β-hydroxysteroid dehydrogenase (17beta-HSD) subtype 1, 3 and 5 compared to CM of normal prostate epithelial cells (RWPE1) (Figure 2). Similar expression profile was detected in two additional pASCs from AA men (SVFA122 and SFVB123) (*data not shown*). The expression of AR, prostate specific antigen (PSA), and AME gene expression by pASCs was corroborated by immunoblotting (Figure 2). In comparison to nASCs, an increase in SRD5A1 and 2 transcripts, which encode for 5α-reductase subtype 1 and 2 required for conversion of T into dihydrotestosterone (DHT), was also observed in four pASC isolates from AA men in response CM of PC cells in a time-dependent manner (Figure 3, next page). The induction of steroidogenic genes was coupled by T production by four pASC isolates pretreated with CM of LNCaP in comparison to controls (next page).

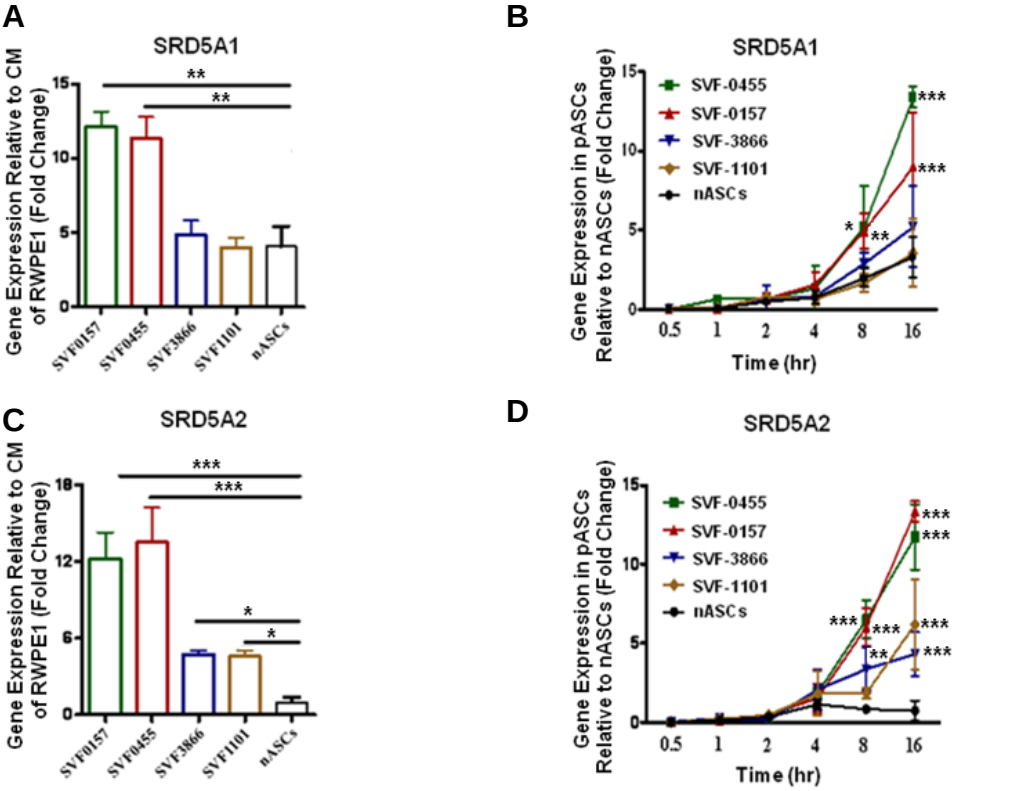

Figure 3: PC cell soluble factors confer transcriptional upregulation of DHT converting enzymes by pASCs *in vitro.* **a**, **b**, Gene expression of DHT converting enzyme 5-alpha-reductase isoforms (SRD5A1 and 2) in four representative pASCs (SVF0157, SVF0455, SVF3866 and SVF1101) exposed to CM derived from PC cells or RWPE1 cells for 24 hr. **c, d**, Temporal expression of SRD5A1 and SRD5A2 genes were monitored in pASCs exposed to CM from PC cells or RWPE1 cells for 0.5 – 16 hrs. Data are expressed as mean ± s.e.m. in triplicate measurements. *, ** and *** denotes significance at p<0.05, p<0.01 and p<0.001 relative to CM of RWPE1 cells or nASCs. (*n*=3).

2.3. *Expose different CaP cells, growing in either complete or charcoal stripped media, to CM from the above ADMSCs and measure CaP cell growth rate by BrDU labeling and CFU-assays.*

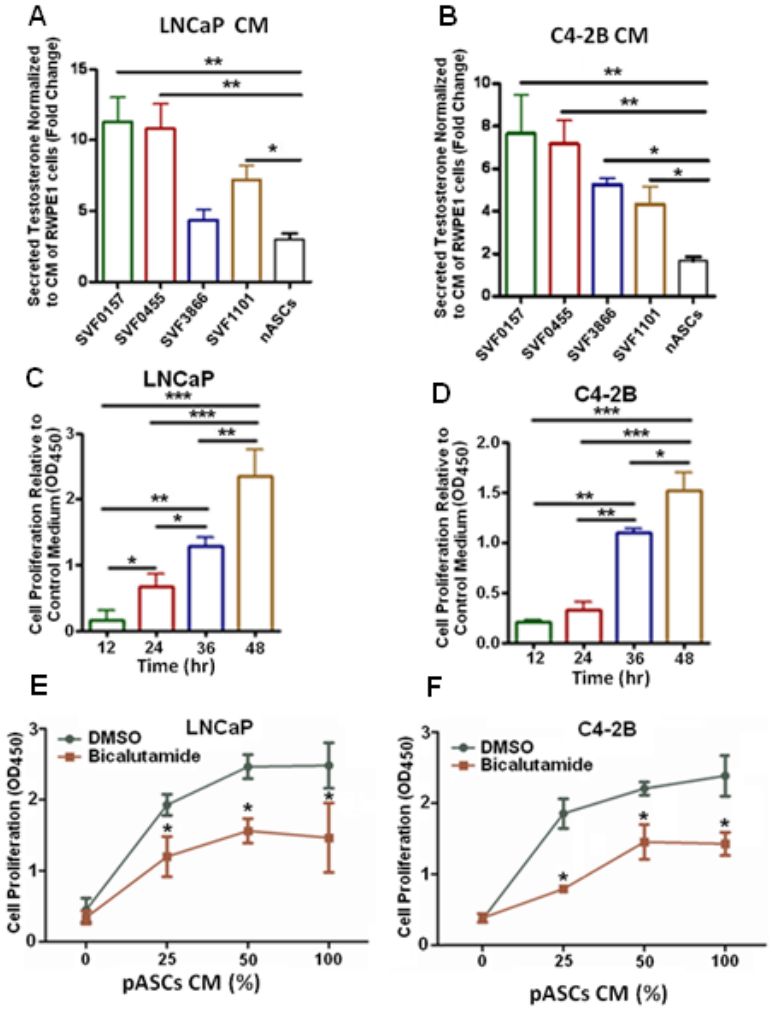

Figure 4 (right): CM of PC cells triggers pASCs to produce androgens and promote growth of PC cells *in vitro*. A, B, nASCs and pASCs cultured in charcoal-stripped media were subjected to CM of LNCaP, C4-2B or RWPE1 cells for 48 hrs. Secreted testosterone by four pASC isolates was quantified in triplicates by an EIA kit relative to the nASC CM after normalization to RWPE1 CM. Time course studies of LNCaP **(C)** and C4-2B **(D)** cell proliferation following exposure to CM (50%) of pre-stimulated pASCs are monitored by WST-8 kit. LNCaP **(E)** and C4-2B **(F)** cells were cultured in increasing concentrations of pASCs CM in presence or absence of bicalutamide (Casodex) (10 μM) or vehicle (DMSO). Cell proliferation was measured in quadruplicates by WST-8 proliferation assay kit (OD 450) after normalization to control medium. The P-values *, ** and *** denotes significance at p<0.05, p<0.01 and p<0.001, respectively, relative to control medium, CM of RWPE1 cells or nASCs. (*n*=3).

The induction of steroidogenic genes was coupled by T production by four pASC isolates pretreated with CM of LNCaP (Fig. 4a) or C4-2B cell (Fig. 4b) in comparison to controls. The CM of ASCs, initially primed with PC cell CM, induced proliferative responses in PC cells in a time-dependent manner (Fig 4 C, D). The growth

stimulation is due to T production by pASCs as evidenced by bicalutamide inhibition (Fig. 4E, F).

2.4. *Coculture ADMSC^{AA} cells with CaP cells (GFP-labeled) and measure percent change in PSA- and/or GFP +ve cells, in the presence or absence of androgen inactivation by recombinant α-HSD.*

These experiments were not completed due to unavailability of recombinant α-HSD from commercial sources. Efforts are currently made to make recombinant α-HSD to complete these experiments.

<u>Task-3</u>: Investigate the *in vivo* effects of ADMSCs coinjected with CaP cells on tumor burden in nude mice and demonstrate the role of ADMSCs in androgen production and tumor growth (Months 5-11).

3.1. *Monitor tumor size in male nude-mice (gonad intact or castrated) subcutaneously injected with CaP cells, alone and/or with ASCs^{AA}, at different time points post xenograft.*

3.2. *In similar in vivo studies using labeled CaP cells (ZsGreen) and labeled ADMSCs (mCherry) harvest tumors and determine steroidogenic enzymes in engrafted ADMSCs by IHC and IFM analysis*.

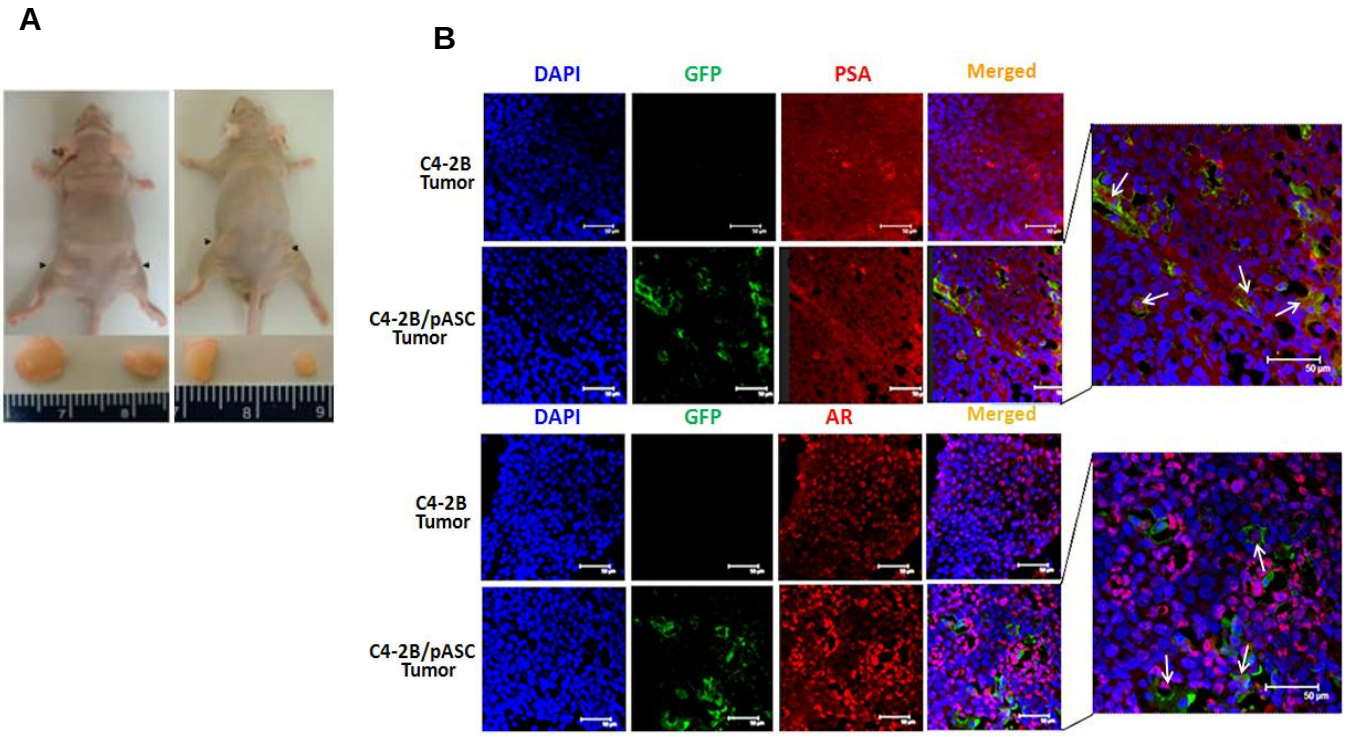

Figure 5. Tumor tropic pASCs from AA men promote PC cell growth *in vivo*. A, Tumor sizes were monitored for 4 weeks by C4-2B cells transplanted alone (right flank) or in combination with pLV-eGFP-labeled tumor-tropic pASCs (left flank) (*arrowhead*) in athymic (*nu/nu*) nude mice. Respective sizes of the resected tumors are shown in the lower panel. **B,** Confocal microscopy images of PC markers AR and PSA are shown in representative immunofluorescent stained tumor sections of C4-2B and pASC/C4-2B xenografts. DAPI stained nuclei are shown in blue. GFP staining (green) indicates engrafted pASC in tumor stroma and AR and PSA are indicated by red fluorescence by individual channels. Insets show magnified merged channels whereas arrows indicate double-positive nuclear and cytoplasmic staining for AR and PSA, respectively, in tumor engrafted pASCs in insets. Scale bars: 50 μm.

To assay for the ability of pASCs from AA men to engraft in PC tumors *in vivo*, the enriched nASCs and pASCs were transduced with a pLV-eGFP and stable clones were selected with puromycin, screened for GFP expression by Nikon E400 fluorescence microscopy (Nikon Instruments, Melville, NY), expanded (passage <5) and stored until used. Next, C4-2B alone or combined with GFP stained or unstained tumor-tropic pASCs from AA-men were transplanted s.c. in nude mice for 4 weeks. Tumor growth and expression of prostate specific markers (AR and PSA) and ARE was examined by immunofluorescence.

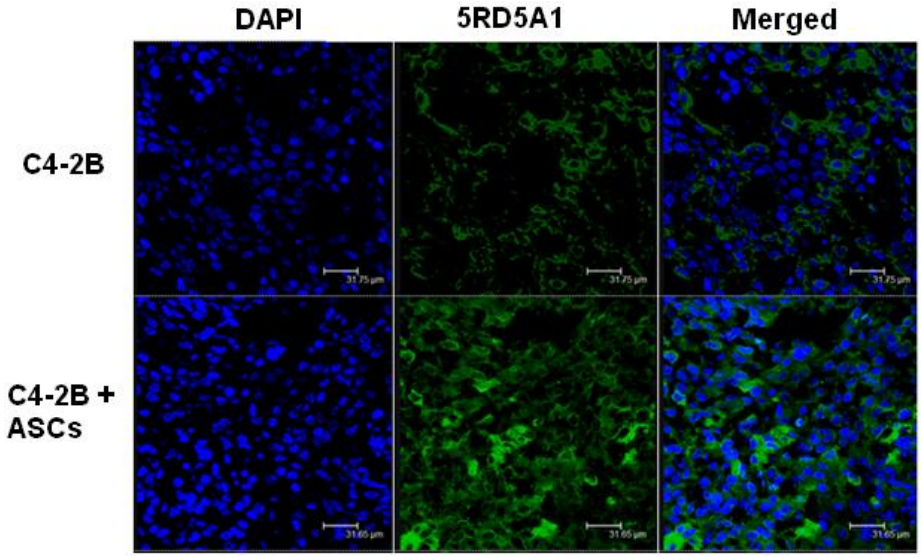

Figure 6: Detection of ARE expression in PC xenografts co-transplanted with pASCs from AA-men. Representative micrographs depicting high expression of 5RD5A1 enzymes, required for conversion of T into DHT, in C4-2b/pASC tumors compared to xenografts developed by C4-2B cell alone.

Briefly, six-week-old athymic BALB/C nude (*nu/nu*) male mice (Harlan, Indianapolis, IN) were housed in a pathogen-free facility and were given free access to commercial rodent chow and water according to approved IACUC animal protocol at Tulane University. To assess the effect of ASCs on PC cell growth *in vivo*, C4-2B cells (1×10^6) mixed with unstained or eGFP-expressing nASCs or pASCs (1×10^5) were suspended in 50 µL of serum-free DMEM medium and an equal volume of Matrigel (BD Bioscience, MD) and subsequently transplanted s.c. by a syringe fitted with a 27-gauge needle into the right and left flanks of each mouse (n=10) for 4 weeks. Control mice were injected with C4-2B, nASCs or pASCs.

Tumors were resected from anesthetized mice after heart perfusion with PBS. Part of the tumors was snap-frozen or paraffin-embedded for further analysis. The frozen sections were fixed in cold acetone/methanol (1/1 v/v) solution, washed thrice with PBS, blocked with 10% of goat serum for 1 hr after washing twice with 0.2% Triton X-100 in PBS, then incubated (4°C, 12–16 h) with primary antibodies against AR, PSA and AMEs Tissue sections were then incubated for 1 hr at room temperature in PBS/0.05% Tween-20 solution containing secondary antibodies conjugated with Alex Flour 488, 594, or 647-conjugated IgGs; (Molecular Probes, Invitrogen, Carlsbad, CA). Slides were mounted with mounting medium with DAPI to stain the nuclei (Vectashield, Burlingame, CA). Images were acquired by confocal Leica TCS SP5 microscope/LAS AF software or Olympus IX70 inverted fluorescence microscope/MagnaFire software.

As shown in Figure 5 (above), pASC engrafted within the tumor parenchyma. In addition, the resulting C4-2B/pASC tumor xenografts were larger (2 to 3-fold) than the wild-type C4-2B tumors in 7 out of 10 mice studied in 4 weeks. Immunofluorescence (IF) analysis demonstrate that the engrafted pASCs express AR and PSA (Figure 5), suggesting that the tumor-tropic pASCs not only engraft and enhance the tumor mass, but also undergo transdifferentiation into prostate tumor-like cells. Moreover, we also demonstrate that ARE, such as 5RD5A1, is also expressed at higher levels by C4-2B/pASC xenogrfats compared to C4-2B alone, suggesting that testosterone production by engrafted pASCs may contribute to promotion of PC cell growth *in vivo*.

Discussion

Emerging evidence suggests that tumor-tropic mesenchymal stem cells (MSCs) promote tumor growth via unknown mechanisms. Herein, we report that prostate cancer cell-derived soluble factors (SF) trigger *in vitro* and *in vivo de novo* synthesis of key endogenous steroidogenic enzymes (Cyp17A, AKR1C3, 3BHSD2 and SRD5A2) in AA patient-derived adipose stem cells (pASCs) that are indispensable for testosterone and DHT production *in vitro*. The androgen production by pASCs is physiologically active and sufficient to support prostate cancer cell growth *in vitro*. Co-transplantation assays demonstrate engraftment and promotion of PC cell growth *in vivo*. The tumor growth was associated with increased

expression of 5RD5A1). Together, our findings propose new concepts for elevated androgen metabolism within the tumor microenvironment by tumor recruited MSCs and their potential role in tumor clonal expansion at primary and metastatic sites. Our observations also suggest that a multi-targeted hormonal therapeutic modality designed to ablate 'intracrine' androgens by pASCs is required to enable effective and optimal treatment response in patients with castration-resistant PC (CRPC), especially among AA-men.

Key Research Accomplishments: The following experiments were completed:

a. Obtained lipoaspirates from normal donors and isolate and culture pASCs by using protocols already established in our laboratories.

b. Analyzed stem cell characteristics in pASCs by flow cytometry and lineage-specific differentiation, and development of large batches of liquid-N_2 stocks for future experiments.

c. Harvested and frozen aliquots of charcoal-stripped conditioned medium (CM) from different CaP cell lines, e.g. LNCaP, C4-2B, PC-3, MDA-PCa-2a and MDA PCa-2b cell lines.

d. Expose ASC^{Cont} and ASC^{AA} cells to CM from the above CaP cells and measure testosterone (T) and dihydrotestosterone (DHT) levels in culture media by ELISA assays.

e. Obtained total RNA and proteins from ASCs, control and CaP CM-exposed, and determine the change in expression of different steroidogenic enzymes by qRT-PCR and Western blot.

f. Monitored engraftment of pASXCs and tumor size in male nude-mice (gonad intact or castrated) subcutaneously injected with CaP cells alone and/or with $ASCs^{AA}$.

g. In similar *in vivo* studies, we also monitored expression of prostate cancer specific markers and ARE in tumor xenografts by immunofluorescence analysis.

Reportable Outcomes:

1. Meeting presentations: The outsome of the studies will be presented at the annual AACR meeting in Wasshington, DC, 2013.

2. Manuscript is under preparation for publication in the foreseeable future.

Conclusions: African Americans (AA) have twice the incidence and mortality of prostate (PC) than Caucasian Americans (CA). While the disproportionate burden was partially explained by genetic, socioeconomic, and environmental factors, racial variation in the biology of prostate tumors was not investigated. “Intracire” androgens are consistently implicated in the outgrowth of castration-adapted prostate tumors through activation of functional androgen-signaling cascade under androgen deprivation therapy (ADT). Emerging evidence suggests that tumor-tropic mesenchymal stem cells (MSCs) promote tumor growth via unknown mechanisms. Herein, we report that prostate cancer cell-derived soluble factors (SF) trigger *in vitro* and *in vivo de novo* synthesis of key endogenous steroidogenic enzymes (Cyp17A, AKR1C3, 3BHSD2 and SRD5A2) in AA patient-derived adipose stem cells (pASCs) that are indispensable for testosterone and DHT production *in vitro*. The androgen production by pASCs is physiologically active and sufficient to support prostate cancer cell growth *in vitro*. Co-transplantation assays demonstrate engraftment and promotion of PC cell growth *in vivo*. The tumor growth was associated with increased expression of 5RD5A1). Together, our findings propose new concepts for elevated androgen metabolism within the tumor microenvironment by tumor recruited MSCs and their potential role in tumor clonal expansion at primary and metastatic sites. Our observations also suggest that a multi-targeted hormonal therapeutic modality designed to ablate 'intracrine' androgens by pASCs is required to enable effective and optimal treatment response in patients with castration-resistant PC (CRPC), especially among AA-men.

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